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**(54) Use of bile salt-activated lipase as dietary supplement**

Verwendung von Gallsäure-aktiviertem Lipase als Diätsupplement

Utilisation de lipase activé de sel biliaire comme supplément diététique

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(56) References cited:  
**EP-A- 0 021 129                      FR-A- 2 313 916  
GB-A- 1 571 877                      US-A- 3 256 150  
US-A- 3 991 180**

- **PEDIATRIC RESEARCH, vol.16, no.10, October 1982 pages 882 - 885 O. HERNELL ET AL. 'Digestion of Human Milk Lipids: Physiologic Significance of sn-2 Monoacylglycerol Hydrolysis by Bile Salt-Stimulated Lipase'**
- **JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, vol.2, 1983, NEW YORK pages 242 - 247 O. HERNELL ET AL. 'Bile-Salt-Stimulated Lipase of Human Milk and Lipid Digestion in the Neonatal period'**

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**Description**Field of the Invention

5 **[0001]** The present invention relates to dietary compositions in general and in particular to dietary compositions and methods using bile salt-activated lipase.

Prior Art

10 **[0002]** US-A-3 991 180 (Boettner *et al*) discloses pancreatic lipases. Hernell & Bläckberg (1982 *Pediatr. Res.* **16**, 882-885) discusses the natural situation when newborn infants are fed human milk. GB-A-1 571 877 discloses a microbial lipase and its use to aid digestion of fats. Alemi *et al* (1981 *Pediatrics* **68**, 484-489) is a study concerning the addition of human milk to milk formula. An article by Jenkins, K.J. in 1979 *Can. J. Anim. Sci.* **59**, 463-465 discloses an in vitro study that indicated that pancreatic lipase can hydrolyse milk replacer fats under conditions stimulating those in  
15 the small intestine. When pancreatic lipase was added to high fat milk replacer very little lipolysis occurred in vitro at the low pH value encountered in the true digestive stomach.

Summary of the Invention

20 **[0003]** The present invention is directed to a dietary composition which comprises a nutritional base from a first source containing fats and being poor in bile salt-activated lipase. The composition further comprises an effective amount of bile salt-activated lipase derived from a second source. The present invention also comprises an infant formula for a first animal species which comprises milk from a second animal species and an effective amount of bile salt-activated lipase derived from a species other than the second animal species.

25 **[0004]** The present invention further comprises a method for fortifying cow's milk. An effective amount of bile salt-activated lipase is added thereto. The lipase is derived from an animal species other than a cow. According to the present invention other fat-containing infant formulas which are poor in bile salt-activated lipase may be similarly fortified by adding an effective amount of bile salt-activated lipase.

30 **[0005]** The present invention further comprises a method for treating a subject for inadequate pancreatic production of bile salt-activated lipase. Bile salt-activated lipase is administered to the subject in conjunction with the ingestion of fats and in an amount sufficient to improve the subject's digestion of the fats.

**[0006]** Finally, the present invention includes a method for feeding an infant. A fat-containing dietary base is administered to the infant. In conjunction therewith, bile salt-activated lipase is administered to the infant in an amount sufficient to improve the infant's digestion of the fats in the base.

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Description of the Preferred Embodiments

**[0007]** In warm-blooded animals, the proper digestion of fat requires a class of digestive enzymes called lipases. Lipases occur in many forms and are produced by several of the digestive organs, including the pancreas, stomach and  
40 small intestines. The present invention concerns a subclass of lipases called bile salt-activated lipases, so named because they are secreted in an inactive form and are activated in the intestines in the presence of bile salts.

**[0008]** Bile salt-activated lipase exists in two forms. One is produced by the pancreas and is referred to as pancreatic bile salt-activated lipase or pancreatic carboxylesterase. The other is found in milk and is referred to as milk bile salt-activated lipase. Because of their functional and chemical similarities, these enzymes represent a pair of isozymes.  
45 They are directed to the same substrate, fatty acid esters, but are released independently into the digestive tract. [Wang and Kloer, *Biochim. Biophys. Acta*, **252**:142-149 (1983)].

**[0009]** Milk bile salt-activated lipase has been found only in the milk of certain species, namely humans, gorillas and recently cats and dogs. [Freed, et al., *Biochim. Biophys. Acta*, **878**:209-215 (1986)]. Milk bile salt-activated lipase is not produced by cows, horses, rats, rabbits, goats, pigs or Rhesus monkeys. [Blackberg, et al., *FEBS Lett.* **112**:51-  
50 54 (1980); Freudenberg, *Experientia* **22**:317 (1966)].

**[0010]** Although milk bile salt-activated lipase and its isozyme pancreatic carboxylesterase have been the subject of considerable study, the physiological significance of these enzymes heretofore has remained unknown. The present invention is based on the discovery that bile salt-activated lipase plays a major rate-limiting role in growth. Accordingly, the present invention is directed to supplementation of dietary compositions, such as infant formulas, with milk bile salt-activated lipase or pancreatic carboxylesterase. Also, pathological conditions involving pancreatic dysfunction, such as  
55 cystic fibrosis, may be treated by administering these enzymes to the subject in conjunction with the ingestion of fats.

**[0011]** In accordance with the present invention, a dietary base from a first source is selected. The composition of the base, the balance of protein, carbohydrates, vitamins, etc., will be designed to supply the nutritional needs of the

subject who will consume it. Among these other ingredients, the base contains fats or triglycerides. The source of the fat also may vary according to the intended purpose of the composition and the subject involved. For example, when the composition is an infant formula, the fat usually will be milk fat.

**[0012]** The nutritional base of this invention is poor in bile salt-activated lipase ("BAL") in that insufficient amounts of the enzyme are present to permit optimum hydrolysis of the fats in the intestines when exposed to bile salts. Indeed, most bases will contain no bile salt-activated lipase.

**[0013]** An important application of the present invention is a fortified infant formula for human infants. Thus, in this embodiment the preferred nutritional base usually will comprise cow's milk, which does not contain BAL. Preferred nutritional bases for this application include commercially prepared cow's milk and commercially prepared infant formulas comprising cow's milk. Suitable formulas include those marketed under the names Enfamil<sup>®</sup> by Mead-Johnson & Company (Evansville, Indiana) and Similac<sup>®</sup> by Ross Laboratories (Columbus, Ohio).

**[0014]** It will be appreciated that in many instances the preferred nutritional base will contain no milk, such as where the composition is an infant formula for an infant who does not tolerate milk well. In such cases, preferred nutritional bases include milk-free infant formulas which usually contain soy protein. These include ProSobee<sup>®</sup> marketed by Mead-Johnson & Company and Isomil<sup>®</sup> marketed by Ross Laboratories.

**[0015]** Having selected a suitable nutritional base for the composition, bile salt-activated lipase next is prepared. Milk BAL or pancreatic carboxylesterase may be used. As will later appear, the enzyme may be purified from natural sources or it may be produced by genetic engineering. When the amino acid sequences of these enzymes or their function fragments have been identified, synthetic BAL or its active fragments may be constructed artificially. It will be understood that through these various technologies, enzymes may be produced which are functionally similar but not structurally identical.

**[0016]** Accordingly, as used herein, the term bile salt-activated lipase includes pancreatic carboxylesterase and milk bile salt-activated lipase as well as functionally equivalent enzymes and fragments thereof, regardless of their source or method of production. An enzyme or fragment is functionally equivalent if it is characterized by being bile salt-activated and capable of hydrolyzing fatty acid esters. Likewise, as used herein, to be derived from these enzymes or their natural sources, such as pancreatic tissue (juice) or milk, means to be produced directly, such as by purification, or indirectly by genetic engineering, protein synthesis or the like.

**[0017]** The BAL used in the composition preferably will be derived from the same animal species as the subject who will consume the composition. For example, where the intended subject is human, the BAL preferably will be derived from a human source, such as human milk or human pancreatic carboxylesterase. However, as indicated, milk BAL is present in the milk of at least three other animal species, namely gorillas, cats and dogs, and the studies described below demonstrate functional similarity and high species cross-reactivity between cat BAL and human BAL. Thus, it will be understood that while BAL from the same species as the subject is preferred, BAL from another species may be employed successfully.

**[0018]** BAL may be isolated from milk and purified for use in accordance with the present invention. A preferred method for isolating BAL from milk is described by Wang and Johnson. [Anal. Biochem. 133:457-461 (1983)]. This method utilizes affinity chromatography wherein the enzyme is collected using a cholate Sepharose support. Lactoferrin is removed using a heparin Sepharose support. Using this technique, about 30 mg of purified enzyme may be obtained from 450 ml of human skim milk. Preferably, the end product will be lyophilized. It then may be used later in the powdered state or resolubilized. If the enzyme is stored in solution, it should be kept under refrigeration.

**[0019]** Alternately and preferably, BAL may be produced using now well established recombinant DNA technology. This artificial production of bile salt-activated lipase will be more economical and efficient than purification processes. Several procedures are suitable. For example, the complement DNA of human BAL may be isolated and used to engineer expression vectors for synthesis of the enzyme in microorganisms, or in mammalian or other cell lines. A source of the messenger RNA of human BAL in human mammary gland cell lines, ATTC No. HBL-100, is available from American Type Culture Collection (Rockville, Maryland). Another source of complement DNA of human BAL is the cDNA libraries of lactating human breast tissue in  $\lambda$  phages ( $\lambda$  gt 10 and  $\lambda$  gt 11) which may be obtained from Clontech Laboratories, Inc. (Palo Alto, California).

**[0020]** As naturally occurring BAL has been isolated, anti-BAL antibodies may be produced and used to find the BAL clones in the expression libraries. Alternately, a partial structure of the purified BAL could be determined and used to design nucleotide probes for finding the BAL clones.

**[0021]** Expression vectors and promoters for *E. coli*, yeast and other microorganisms are available for synthesizing BAL. Also, expression systems, such as viral and mammalian promoters and mammalian cell lines, are commercially available for engineering BAL expression systems in other cell lines.

**[0022]** Transgenic animal technology offers another means for synthesizing BAL. The cloned human BAL gene first can be isolated from genomic DNA and then transferred into another animal or mammalian cell line for synthesis of the enzyme. Human genomic libraries are commercially available or may be prepared easily in most laboratories. Complement DNA for human BAL can be isolated as described above and used as a probe to find the BAL gene in the genomic

libraries. The gene transfer can be accomplished by known techniques. For example, gene transfers into mice and other animals has been employed successfully by using microinjections of foreign genes into fertilized eggs.

**[0023]** By this transgenic technology, other animals, such as cows, can be caused to produce milk which contains human BAL. This will not only produce large amounts of the enzyme, but will produce it in a milk base. If a dietary composition of this invention is produced in this manner, it will be understood that the milk should not be subjected to heat, i.e. in excess of 50° C, as this will reduce the activity of the enzyme. In particular, methods of preparing the milk should not include conventional boiling procedures.

**[0024]** Pancreatic carboxylesterase may be isolated from pancreatic juice by affinity chromatography as described by Wang and Kloer. [Biochim. Biophys. Acta, 754:142-149 (1983)]. However, as with milk BAL, it is expected that synthesis, such as by recombinant DNA or transgenic technologies described above, will provide a more abundant and economical source of this enzyme.

**[0025]** Having obtained BAL in a substantially purified state, the BAL is added to the nutritional base. Because the preferred base is in a liquid form, i.e. milk or formula, the BAL may be added to the base in its powdered form. Alternately, the BAL may be resolubilized, such as in water or some other non-acidic solution, and then mixed with the base.

**[0026]** The BAL is added in an amount sufficient to improve hydrolysis of the fats in the base by the subject. Where the subject is of a species whose milk contains BAL, such as humans, the amount of BAL normally present in the milk of that species may be used as a guide. For example, it has been shown that human milk contains about 0.1 mg BAL per/ml of human skim milk. Accordingly, the preferred concentration of BAL in 1 ml of this invention would be at least about 0.001 mg, more preferably at least about 0.05 mg and most preferably about 0.1 mg. Alternately, the lipase should be present in the composition in at least a 1:2000 ratio (by weight) to the amount of fats in the composition, more preferably in a ratio of at least 1:1000, and most preferably about 1:200. That is, a preferred amount of lipase is 1 mg for every 200 mg of fat in the composition.

**[0027]** In accordance with the present invention, a method is provided for fortifying cow's milk. Bile salt-activated lipase, derived from an animal species other than cow, is added in an effective amount to the milk. The BAL may be obtained or prepared as described above. As indicated above, an effective amount is at least enough to improve hydrolysis of fats in the milk when the milk is digested by a subject, such as a human infant. Where the subject is human, the preferred BAL will comprise human pancreatic carboxylesterase or human milk BAL, and most preferably the latter. The preferred concentration is about 0.1 mg BAL per 1 ml milk, or about 1 mg of BAL per 200 mg of fat.

**[0028]** Similarly, the present invention provides a method for fortifying a fat-containing infant formula which is poor in bile salt-activated lipase. The formula preferably comprises cow's milk, but alternately may be a non-milk based formula. BAL is added to the formula in an effective amount as described previously. The BAL preferably is derived from an animal of the same species as the infant. Alternately, pancreatic carboxylesterase may be used, also preferably derived from the same species as the infant to consume it.

**[0029]** The present invention further relates to a method for treating a subject for inadequate pancreatic production of bile salt-activated lipase, and in particular pancreatic carboxylesterase. Such conditions occur in subjects suffering from pancreatic disease or trauma resulting in reduced fat absorption. Also, genetic disorders, such as cystic fibrosis, may affect enzyme production by the pancreas, and thus, fat absorption. The compositions of the present invention may be used in the treatment of these maladies. In accordance with this method, BAL is administered to the subject in conjunction with the ingestion of fats by the subject. The BAL may be pancreatic carboxylesterase or milk BAL and may be administered in powdered or liquid form. It may be mixed with the subject's food or drink, or may be given in an enteric coated capsule or tablet. It may be given shortly before or after the meal, or with the meal.

**[0030]** The BAL is administered in an amount sufficient to improve the subject's absorption or digestion of the ingested fats. It should be noted that the level of pancreatic function may vary among subjects in need of this therapy. Thus, fat absorption studies may be desirable to determine more precisely the amount of BAL required to achieve optimum fat absorption in a particular subject.

**[0031]** It will be understood that some of the above mentioned disorders also involve an absence or deficiency of bile salt production. In such case, successful treatment in accordance with the present invention may require concomitant administration of bile salts. When this is necessary, the bile salts and the enzyme should not be allowed to react with the fats prior to their ingestion, as this will result in unpalatable flavor.

**[0032]** Although it has not been confirmed, it is believed that in human infants and particularly in premature infants, pancreatic development and therefore enzyme production is incomplete at birth. The presence of BAL in human milk is thought to be a compensatory mechanism for the infant's immature pancreatic function. However, many human infants are not breast fed, and the most common infant formula comprises cow's milk which does not contain milk BAL.

**[0033]** Thus, the present invention provides an improved method for feeding infants. A dietary base from a first source and comprising fats is administered to the infant. The base preferably comprises cow's milk or a prepared infant formula comprising cow's milk. However, if necessary, a milk-free formula with the necessary fats may be employed.

**[0034]** BAL from a second source also is administered to the infant in an amount sufficient to improve the infant's digestion of fats in the formula, and most preferably at the rate of about 1 mg per 200 mg fat in the base. Preferably, the

BAL is obtained from the same species as the infant, so that human BAL, preferably milk BAL, is given to human infants. The BAL may be administered with the base or shortly before or after it.

EXAMPLES

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1. MILK BILE SALT-ACTIVATED LIPASE ACTIVITY STUDIES OF HUMAN AND CAT MILK

[0035] Recently Freed, et al. reported the presence of bile salt-activated lipase in the milk of cats and dogs. [Biochim. Biophys. Acta, 878:209-215 (1986)]. We performed studies to confirm this.

10 [0036] Cat milk was obtained from 4 common cats. The milk was expressed using a device similar to that described by McKenzie and Anderson [J. Dairy Sci., 62:1469-1470 (1979)] for milking small animals. Samples of fifteen day post partum human milk was obtained from Oregon Health Science Center (Portland, Oregon) and shipped in dry ice.

15 [0037] Samples of cat milk and human milk were tested for lipase activity using trioleoylglycerol as a substrate according to the procedure described by Wang, et al. [Clin. Chem. 27: 663-668 (1981)]. All assays were carried out in a NH<sub>4</sub>OH-HCl buffer solution at a pH of 8.5. The substrate (trioleoylglycerol) concentration was 10 μmol/ml and 0.1 μCi/mol of the labeled substrate, namely glycerol tri[C<sup>14</sup>] oleate (from Amersham Corp. of Arlington Heights, Illinois). The concentration of bovine serum albumin was 60mg/ml of solution, and the concentration of the activator taurocholate (bile salt) was 20 mM of solution. The final volume of the assay mixture of 1.0 ml.

20 [0038] After incubation of the mixture at 37° C in a shaking water bath for 1 hour, the reaction was stopped by adding 4 ml of a mixture of isopropanol and 1.5 M H<sub>2</sub>SO<sub>4</sub> (40:1, v/v). After mixing, the lipids were extracted by further addition of 2 ml water and 5 ml of hexane, and shaking with a votex. Fatty acids were extracted from 3 ml of the separated hexane layer with 1 ml of 0.2 M KOH. A 0.5 ml aliquot of the aqueous KOH solution was taken for measurement of the radioactivity in the usual manner on a scintillation counter. One unit of activity was defined as the amount of enzyme catalyzing the release of 1 μmol of fatty acid per hour at 37° C. The results are shown in Table 1.

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TABLE I

BILE SALT-ACTIVATED LIPASE ACTIVITY IN CAT AND HUMAN MILK SAMPLES										
Cat Milk							Human Milk			
	3 days Postpartum		6 days Postpartum		12-18 days Postpartum			15 days Postpartum		
	u*/ml	cpm	u/ml	cpm	u/ml	cpm		u/ml	cpm	
35	Sample 1	439	5869	341	5339	224	3176	Sample 1	556	3521
	Sample 2	169	2673	205	3229	183	2465	Sample 2	550	3483
	Sample 3	536	8377	281	4567	244	3839	Sample 3	615	3888
40	Sample 4	---	----	---	----	146	2391	Sample 4	665	4199
								Sample 5	599	3791
								Sample 6	358	2278
45	Average	381	----	275	----	199	----		557	----
	SEM	110		39		122			43	
	N	3		3		4			6	

\*Values expressed are units of activity. One unit is defined as the amount of enzyme catalyzing the release of 1 μmol of fatty acid per hour at 37° C.

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[0039] The data in Table I confirmed previous reports on bile salt-activated lipase. It was also observed that the mean activity of this enzyme in cat milk declines with the length of the lactation period. However, this decrease is believed to be insignificant statistically because of the wide range of enzyme activity among the animals tested. Human milk contains about twice the enzyme activity as did cat milk.

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## 2. COMPARATIVE IMMUNOCHEMICAL ACTIVITY OF CAT AND HUMAN ENZYME

5 [0040] The functional similarity of cat BAL and human BAL, that is that both enzymes hydrolyze fats, has been previously reported. [Freed, et al., Biochim. Biophys. Acta, 878:209-215 (1986)]. However, the immunochemical similarity of these enzymes had not been demonstrated. Accordingly, we next examined the cross-reactivity of human BAL and cat BAL by performing antibody inhibition studies.

10 [0041] Antibodies against human bile salt-activated lipase were prepared from a rabbit. The antibodies in the antiserum from the rabbit was collected and purified using affinity chromatography. Specifically, we used an affinity column loaded with covalently linked purified human BAL and Sepharose 4B. 3 M NaSCN was used to elute the retained antibodies. The monospecific antibodies then were used in a lipase assay procedure to test reactivity of the antibodies with human milk bile salt-activated lipase and with cat milk bile salt-activated lipase. The results are shown in Table II.

TABLE II

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EFFECT OF HUMAN MILK BAL ANTIBODIES ON BAL ACTIVITY IN HUMAN MILK, CAT MILK AND ANTIBODY-FREE SERUM			
Control (Non-BAL immunized rabbit serum gamma globulin)			
Antibody Aliquots (ml)	% Activity Remaining	CPM*	BAL Activity**
0.000	100.0	2001	290.29
0.025	100.9	2019	292.90
0.050	99.6	1994	289.29
0.100	97.8	1957	283.91
0.150	98.0	1961	284.45
0.200	96.9	1938	281.15
Cat Milk			
Antibody Aliquots (ml)	% Activity Remaining	CPM*	BAL Activity**
0.000	100.0	2001	290.29
0.025	87.3	1747	253.44
0.050	74.5	1491	216.30
0.100	45.4	908	131.73
0.150	32.9	659	95.60
0.200	23.7	475	68.91
Human Milk			
Antibody Aliquots (ml)	% Activity Remaining	CPM*	BAL Activity**
0.000	100.0	993	144.06
0.025	41.1	408	59.19
0.050	15.0	149	21.62
0.100	5.4	54	7.83
0.150	5.1	51	7.40
0.200	3.0	30	4.35

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\*Value reflects correction background CPM reading which equaled 42.

\*\*Expressed in units, one unit being defined as the amount of enzyme catalyzing the release of 1  $\mu$ mol of fatty acid per hour at 37° C.

55 [0042] As Table II shows, antibodies against bile salt-activated lipase from human milk inhibited enzyme activity in both cat milk and human milk. However, the human enzyme antibodies were only about 70% as reactive with the cat enzyme as with the human enzyme. From this we concluded that cat and human milk BAL are strongly cross-reactive

between the species, but are not identical.

3. COMPARATIVE GROWTH STUDIES

5 **[0043]** Having verified that cat's milk contained bile salt-activated lipase and that human BAL was immunochemically similar to cat BAL, kittens were chosen as an ideal animal model to study the digestive function of this enzyme using purified human BAL. Three pregnant cats of common variety were selected and allowed to deliver their litters normally. Each litter had six kittens (two died). All of the kittens from each litter were allowed to breast feed normally for 48 hours after birth to ensure survival. At the end of this initial breast feeding period, the five-day feeding experiment was  
10 begun.

**[0044]** From each of the three litters, four kittens were selected arbitrarily -- two males and two females. One male and one female from each litter was used as control; and similarly, one male and one female from each group were used as the study group. The rest of the kittens were allowed to continue breast feeding throughout the five-day study. However, two of the remaining six kittens died, so the breast fed group comprised four kittens.

15 **[0045]** The six kittens in the control group were fed a mixture of commercially prepared kitten formula (Borden® Liquid KMR® Milk Replacer for Kittens manufactured by Pet-AG, Inc. of Hampshire, Illinois) mixed three parts to one part with commercially prepared homogenized vitamin cow's milk (Borden's® Homogenized Vitamin D milk). The six kittens in the study group were fed the same formula except that it was supplemented with purified human bile salt-activated lipase at a concentration of 0.1 mg lipase per 1 ml of formula. The lipase used was purified from human milk as  
20 described by Wang, et al. [Anal. Biochem. 133:457-461 (1983)].

**[0046]** Each kitten was fed manually at the rate of 2 ml formula every hour for 5 days. The formula was prepared fresh daily. Each kitten was weighed daily at about the same time, and the weights were recorded. The results are shown in Table III.

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TABLE III

COMPARATIVE GROWTH STUDIES OF KITTENS								
Breast fed group	(wt. in gms)	(wt. gain in gms/day)					Average wt. gain per day	
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5		
Kitten #1	114.0	13.3	7.3	10.8	7.2	9.1	9.54	
Kitten #2	110.0	8.3	11.0	14.3	8.1	10.8	10.50	
Kitten #3	114.9	23.1	14.0	15.0	14.5	8.5	15.02	
Kitten #4	125.0	25.1	15.4	29.0	13.8	6.7	18.00	
Avg. Daily Wt. Gain								13.27
SEM								1.97
Study Group (for- mula + enzyme)								
Kitten #1	82.5	13.0	18.6	7.0	9.2	8.2	11.20	
Kitten #2	75.6	14.5	20.9	3.0	4.3	2.7	9.08	
Kitten #3	109.9	22.9	13.9	11.6	11.4	5.5	13.06	
Kitten #4	109.6	15.6	13.6	10.8	6.6	6.1	10.54	
Kitten #5	103.4	14.1	21.3	4.0	7.1	12.8	11.86	
Kitten #6	96.1	19.7	16.0	6.7	10.5	9.9	12.56	
Avg. Daily Wt. Gain								11.38
SEM								0.59
Control Group (for- mula only)								
Kitten #1	100.6	4.7	9.0	2.5	1.1	1.4	3.74	
Kitten #2	95.0	6.8	2.9	18.6	7.4	8.9	8.92	
Kitten #3	115.5	5.2	5.8	3.1	3.8	3.8	4.34	
Kitten #4	114.1	8.3	3.0	3.6	5.2	5.8	5.18	
Kitten #5	101.2	10.9	14.3	6.6	6.9	0.1	7.76	
Kitten #6	106.5	19.6	6.1	7.4	5.8	5.3	8.84	
Avg. Daily Wt. Gain								6.46
SEM								0.95

[0047] As the information in Table III shows, the kittens in the breast fed group grew at an average rate of 13.3 +/- 2.0 gm/day. The kittens in the study group (those receiving formula supplemented with enzyme) grew at nearly the same rate -- 11.4 +/- 0.6 gm/day. On the other hand, the control group (those fed with unsupplemented formula) grew at less than half that rate, 6.5 +/- 1.0 gm/day. From these results we concluded that bile salt-activated lipase definitely represents the major rate-limiting factor in fat absorption and therefore growth of kittens. Having established the immunochemical similarity of human and cat BAL, we further concluded that BAL plays an equally important role in the growth of human infants.

[0048] Based on the foregoing, it will be appreciated that the present invention provides a simple, inexpensive and accessible dietary supplement to improve digestion of fats and the overall nutritional status. Among many important applications is the preferred embodiment of this invention which relates to formulas and feeding methods for human infants.

**Claims**

**Claims for the following Contracting States : AT, BE, CH, LI, DE, FR, GB, GR, IT, LU, NL, SE**

- 5 1. The use of an isolated mammalian bile salt-activated lipase as the sole added lipase in the manufacture of a dietary supplement for the improvement of the absorption or digestion of ingested fats in a premature baby or in the manufacture of a medicament for treating a patient with a pathological condition involving pancreatic dysfunction wherein the lipase is provided in a dosage providing at least 1 mg lipase/2000 mg ingested fat.
- 10 2. The use according to Claim 1 wherein the pathological condition is cystic fibrosis.
3. The use according to Claim 1 or 2 wherein the patient to be treated is administered bile salts concomitantly with the administration of the medicament.
- 15 4. The use of any one of Claims 1 to 3 wherein the lipase is provided in a dosage providing 1 mg lipase/200 mg ingested fat.
5. The use of any one of the preceding claims wherein the lipase is human milk bile activated lipase, gorilla milk bile activated lipase, dog milk bile activated lipase, cat milk bile activated lipase or pancreatic carboxylesterase.
- 20 6. The use of any one of the preceding claims wherein there is an enteric coating encapsulating the lipase.
7. The use of any one of the preceding claims wherein the concentration of the lipase in the supplement or medicament is greater than 0.1 mg lipase/ml.

25 **Claims for the following Contracting State : ES**

1. The use of an isolated mammalian bile salt-activated lipase as the sole added lipase in the manufacture of a dietary supplement for the improvement of the absorption or digestion of ingested fats in a premature baby or in the manufacture of a medicament for treating a patient with a pathological condition involving pancreatic dysfunction wherein the lipase is provided in a dosage providing at least 1 mg lipase/2000 mg ingested fat.
- 30 2. The use according to Claim 1 wherein the pathological condition is cystic fibrosis.
- 35 3. The use according to Claim 1 or 2 wherein the patient to be treated is administered bile salts concomitantly with the administration of the medicament.
4. The use of any one of Claims 1 to 3 wherein the lipase is provided in a dosage providing 1 mg lipase/200 mg ingested fat.
- 40 5. The use of any one of the preceding claims wherein the lipase is human milk bile activated lipase, gorilla milk bile activated lipase, dog milk bile activated lipase, cat milk bile activated lipase or pancreatic carboxylesterase.
6. The use of any one of the preceding claims wherein there is an enteric coating encapsulating the lipase.
- 45 7. The use of any one of the preceding claims wherein the concentration of the lipase in the supplement or medicament is greater than 0.1 mg lipase/ml.
8. Process for the manufacture of a dietary supplement or a medicament, directed to improve the absorption or digestion of fats ingested by a human being, characterised by comprising the addition of an isolated mammalian bile salt-activated lipase, as the sole added lipase, to a carrier or excipient able for human ingestion, at a rate over 0.1mg lipase/ml of carrier or excipient.
- 50 9. Process according to Claim 8, characterised because said human being is a premature baby.
- 55 10. Process according to Claim 8, characterised because said human being is a patient with a pathological condition involving pancreatic dysfunction.

11. Process according to Claim 8, characterised because the pathological condition is cystic fibrosis.
12. Process according to Claim 10, characterised because said dietary supplement or medicament is provided in a dosage allowing the administration of at least 1mg lipase/2000 mg ingested fat.
- 5 13. Process according to Claim 12, characterised because said dietary supplement or medicament is provided in a dosage allowing the administration of at least 1mg lipase/200mg ingested fat.
- 10 14. Process according to any of the previous Claims 8 to 13, characterised because said dietary supplement or medicament can be administered jointly with bile salts.
- 15 15. Process according to any of the previous claims, where the lipase is human milk bile activated lipase, gorilla milk bile activated lipase, dog milk bile activated lipase, cat milk bile activated lipase or pancreatic carboxylesterase.
16. Process according to any of the previous claims, characterised because the lipase is encapsulated into an enteric coating.

### Patentansprüche

#### 20 Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, FR, GB, GR, IT, LU, NL, SE

- 25 1. Verwendung einer isolierten Säugetier-Gallensäuresalzaktivierten Lipase als einziger zugesetzter Lipase bei der Herstellung einer Diät- bzw. Nahrungsergänzung zur Verbesserung der Absorption oder Verdauung aufgenommener Fette bei einem Frühgeborenen oder bei der Herstellung eines Medikaments zur Behandlung eines Patienten mit einem pathologischen Leiden unter Beteiligung einer Bauchspeicheldrüsenfunktionsstörung, wobei die Lipase in einer Dosis bereitgestellt wird, die für mindestens 1 mg Lipase/2000 mg an aufgenommenem Fett sorgt.
- 30 2. Verwendung nach Anspruch 1, wobei das pathologische Leiden zystische Pankreasfibrose ist.
- 3 3. Verwendung nach Anspruch 1 oder 2, wobei der zu behandelnde Patient gleichzeitig mit der Verabreichung des Medikaments Gallensäuresalze (verabreicht) erhält.
4. Verwendung nach einem der Ansprüche 1 bis 3, wobei die Lipase so dosiert wird, daß pro 200 mg an aufgenommenem Fett 1 mg Lipase zur Verfügung steht.
- 35 5. Verwendung nach einem der vorhergehenden Ansprüche, wobei es sich bei der Lipase um Humanmilch-Gallensäuresalz-aktivierte Lipase, Gorillamilch-Gallensäuresalzaktivierte Lipase, Hundemilch-Gallensäuresalz-aktivierte Lipase, Katzenmilch-Gallensäuresalz-aktivierte Lipase oder pankreatische Carboxylesterase handelt.
- 40 6. Verwendung nach einem der vorhergehenden Ansprüche, wobei die Lipase in einen enterischen Überzug eingekapselt ist.
7. Verwendung nach einem der vorhergehenden Ansprüche, wobei die Konzentration der Lipase in dem Ergänzungsmittel oder Medikament über 0,1 mg Lipase/ml liegt.

#### 45 Patentansprüche für folgenden Vertragsstaat : ES

- 50 1. Verwendung einer isolierten Säugetier-Gallensäuresalzaktivierten Lipase als einziger zugesetzter Lipase bei der Herstellung einer Diät- bzw. Nahrungsergänzung zur Verbesserung der Absorption oder Verdauung aufgenommener Fette bei einem Frühgeborenen oder bei der Herstellung eines Medikaments zur Behandlung eines Patienten mit einem pathologischen Leiden unter Beteiligung einer Bauchspeicheldrüsenfunktionsstörung, wobei die Lipase in einer Dosis bereitgestellt wird, die für mindestens 1 mg Lipase/2000 mg an aufgenommenem Fett sorgt.
- 55 2. Verwendung nach Anspruch 1, wobei das pathologische Leiden zystische Pankreasfibrose ist.
3. Verwendung nach Anspruch 1 oder 2, wobei der zu behandelnde Patient gleichzeitig mit der Verabreichung des Medikaments Gallensäuresalze (verabreicht) erhält.

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4. Verwendung nach einem der Ansprüche 1 bis 3, wobei die Lipase so dosiert wird, daß pro 200 mg an aufgenommenem Fett 1 mg Lipase zur Verfügung steht.
- 5 5. Verwendung nach einem der vorhergehenden Ansprüche, wobei es sich bei der Lipase um Humanmilch-Gallensäuresalz-aktivierte Lipase, Gorillamilch-Gallensäuresalzaktivierte Lipase, Hundemilch-Gallensäuresalz-aktivierte Lipase, Katzenmilch-Gallensäuresalz-aktivierte Lipase oder pankreatische Carboxylesterase handelt.
6. Verwendung nach einem der vorhergehenden Ansprüche, wobei die Lipase in einen enterischen Überzug eingekapselt ist.
- 10 7. Verwendung nach einem der vorhergehenden Ansprüche, wobei die Konzentration der Lipase in dem Ergänzungsmittel oder Medikament über 0,1 mg Lipase/ml liegt.
- 15 8. Verfahren zur Herstellung eines Diät- oder Nahrungsergänzungsmittels oder eines zur Verbesserung der Absorption oder Verdauung von durch einen Menschen aufgenommenen Fetts bestimmten Medikaments, gekennzeichnet durch den Zusatz einer isolierten Säugetier- Gallensäuresalz-aktivierten Lipase als einzigem Lipasezusatz zu einem von Menschen verdaubaren Träger oder Streckmittel in einer Menge über 0,1 mg Lipase/ml Träger oder Streckmittel.
- 20 9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß es sich bei dem Menschen um eine Frühgeburt handelt.
10. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß es sich bei dem Menschen um einen Patienten mit einem pathologischen Leiden unter Beteiligung von Bauchspeicheldrüsenfunktionsstörung handelt.
- 25 11. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß es sich bei dem pathologischen Leiden um zystische Pankreasfibrose handelt.
12. Verfahren nach Anspruch 10, dadurch gekennzeichnet, daß das Diät- bzw. Nahrungsergänzungsmittel oder Medikament in einer Dosis bereitgestellt wird, welche die Verabreichung von mindestens 1 mg Lipase/2000 mg an aufgenommenem Fett gestattet.
- 30 13. Verfahren nach Anspruch 12, dadurch gekennzeichnet, daß das Diät- bzw. Nahrungsergänzungsmittel oder Medikament in einer Dosis bereitgestellt wird, welche die Verabreichung von mindestens 1 mg Lipase/200 mg an aufgenommenem Fett gestattet.
- 35 14. Verfahren nach einem der vorhergehenden Ansprüche 8 bis 13, dadurch gekennzeichnet, daß das Diät- bzw. Nahrungsergänzungsmittel oder Medikament gemeinsam mit Gallensäuresalzen verabreicht werden kann.
- 40 15. Verfahren nach einem der vorhergehenden Ansprüche, wobei es sich bei der Lipase um Humanmilch-Gallensäuresalz-aktivierte Lipase, Gorillamilch-Gallensäuresalzaktivierte Lipase, Hundemilch-Gallensäuresalz-aktivierte Lipase, Katzenmilch-Gallensäuresalz-aktivierte Lipase oder pankreatische Carboxylesterase handelt.
- 45 16. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Lipase in einen enterischen Überzug eingekapselt ist.

### Revendications

#### Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, FR, GB, GR, IT, LU, NL, SE

- 50 1. Utilisation d'une lipase mammalienne isolée, activée par des sels biliaires, en tant que l'unique lipase abutée dans la préparation d'un complément diététique pour l'amélioration de l'absorption ou de la digestion de graisses ingérées chez un nourrisson prématuré, ou dans la préparation d'un médicament destiné au traitement d'un patient présentant un état pathologique impliquant un dysfonctionnement pancréatique, la lipase étant fournie en une dose procurant au moins 1 mg de lipase/2 000 mg de graisse ingérée.
- 55 2. Utilisation selon la revendication 1, dans laquelle l'état pathologique est la fibrose kystique.
3. Utilisation selon la revendication 1 ou 2, dans laquelle on administre au patient à traiter des sels biliaires simulta-

nément à l'administration du médicament.

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4. Utilisation selon l'une quelconque des revendications 1 à 3, dans laquelle la lipase est fournie en une dose procurant environ 1 mg de lipase/200 mg de graisse ingérée.
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5. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la lipase est de la lipase activée par des sels biliaires de lait humain, de la lipase activée par des sels biliaires de lait de gorille, de la lipase activée par des sels biliaires de lait de chienne, de la lipase activée par des sels biliaires de lait de chatte ou de la carboxylestérase pancréatique.
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6. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la lipase est encapsulée dans un enrobage entérique.
7. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la concentration de la lipase dans le complément ou le médicament est supérieure à 0,1 mg de lipase/ml.

**Revendications pour l'Etat contractant suivant : ES**

- 20
1. Utilisation d'une lipase mammalienne isolée, activée par des sels biliaires, en tant que l'unique lipase ajoutée dans la préparation d'un complément diététique pour l'amélioration de l'absorption ou de la digestion de graisses ingérées chez un nourrisson prématuré, ou dans la préparation d'un médicament destiné au traitement d'un patient présentant un état pathologique impliquant un dysfonctionnement pancréatique, la lipase étant fournie en une dose procurant au moins 1 mg de lipase/2 000 mg de graisse ingérée.
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2. Utilisation selon la revendication 1, dans laquelle l'état physiologique est la fibrose kystique.
3. Utilisation selon la revendication 1 ou 2, dans laquelle on administre au patient à traiter des sels biliaires simultanément à l'administration du médicament.
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4. Utilisation selon l'une quelconque des revendications 1 à 3, dans laquelle la lipase est fournie en une dose procurant 1 mg de lipase/200 mg de graisse ingérée.
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5. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la lipase est de la lipase activée par des sels biliaires de lait humain, de la lipase activée par des sels biliaires de lait de gorille, de la lipase activée par des sels biliaires de lait de chienne, de la lipase activée par des sels biliaires de lait de chatte ou de la carboxylestérase pancréatique.
- 40
6. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la lipase est encapsulée dans un enrobage entérique.
7. Utilisation selon l'une quelconque des revendications précédentes, dans laquelle la concentration de la lipase dans le complément ou le médicament est supérieure à 0,1 mg de lipase/ml.
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8. Procédé pour la préparation d'un complément diététique ou d'un médicament destiné à améliorer l'absorption ou la digestion de graisses ingérées par un être humain, caractérisé en ce qu'il comprend l'addition d'une lipase mammalienne isolée, activée par des sels biliaires, en tant que l'unique lipase ajoutée, à un véhicule ou excipient approprié à l'ingestion humaine, à un taux supérieur à 0,1 mg de lipase/ml de véhicule ou excipient.
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9. Procédé selon la revendication 8, caractérisé en ce que ledit être humain est un nourrisson prématuré.
10. Procédé selon la revendication 8, caractérisé en ce que ledit être humain est un patient présentant un état pathologique impliquant un dysfonctionnement pancréatique.
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11. Procédé selon la revendication 8, caractérisé en ce que l'état pathologique est la fibrose kystique.
12. Procédé selon la revendication 10, caractérisé en ce que ledit complément diététique ou médicament est fourni en une dose permettant l'administration d'au moins 1 mg de lipase/2 000 mg de graisse ingérée.

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**13.** Procédé selon la revendication 12, caractérisé en ce que ledit complément diététique ou médicament est fourni en une dose permettant l'administration d'au moins 1 mg de lipase/200 mg de graisse ingérée.

5 **14.** Procédé selon l'une quelconque des revendications précédentes 8 à 13, caractérisé en ce que ledit complément diététique ou médicament peut être administré conjointement avec des sels biliaires.

**15.** Procédé selon l'une quelconque des revendications précédentes, dans lequel la lipase est de la lipase activée par des sels biliaires de lait humain, de la lipase activée par des sels biliaires de lait de gorille, de la lipase activée par des sels biliaires de lait de chienne, de la lipase activée par des sels biliaires de lait de chatte ou de la carboxyl-  
10 térase pancréatique.

**16.** Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que la lipase est encapsulée dans un enrobage entérique.

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